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14. ABSTRACT
Autonomic dysreflexia (AD) is a potential life threatening condition characterized as episodic vascular hypertension (often with bradycardia) that develops in most people with a spinal cord injury (SCI) above thoracic spinal level T5. Using telemetric recording we were able to detect biphasic spontaneous AD developed in mice with T3 SCI; the early phase of AD occurs within first week which is likely due to loss of descending control of sympathetic outflow and the late phase occurs weeks post injury which is likely caused by the formation of aberrant sympathetic neural circuits at the site of injury. We proposed that post-injury inhibition of reactive synaptogenesis would block the onset or reduce the severity of AD. In this study we tested this hypothesis by using both genetic modified mice lines (α2δ-1 over-expressing and TSP KO) and the drug, Gabapentin, which disrupting the binding of α2δ-1 with TSP, to block the formation of aberrant sympathetic nerve circuits and prevent occurring of AD. Current study suggested that mice carry an extra α2δ-1 gene developed more AD than WT littermates after T3 SCI. GBP s.c. (200mg/kg TID) treatment inhibits AD development in WT BL6 mice. Preliminary data from IHC study suggested GBP may promotes inhibitory synapse formation rather than reduce excitatory synapse formation. This study suggested that post-injury synaptogenesis is an important mechanism underlining the development of AD post T3 SCI.

15. SUBJECT TERMS
Spinal cord injury (SCI); Autonomic dysreflexia (AD); Synaptogenesis; Sympathetic pre-ganglionic neurons (SPNs); Gabapentin (GBP); Thrombospondins (TSP); Calcium channel subunit a1d2.

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INTRODUCTION

Autonomic dysreflexia (AD) is a life threatening condition of episodic vascular hypertension (often with bradycardia, i.e., slowed heart rate) that develops in most (~90%) people with a spinal cord injury (SCI) above thoracic spinal level T5. After high level SCI, loss of supraspinal control together with aberrant collateral sprouting and formation of new intraspinal synapses (i.e., synaptogenesis) causes spinal autonomic reflexes to become exaggerated [1-4]. This post-injury maladaptive neural plasticity, involving sensory axons and propriospinal interneurons that connect multiple segments of the thoracic and upper lumbar spinal cord, progresses slowly over the course of several weeks or months post-injury. Prevention (e.g., regular bladder/bowel care) and anti-hypertensive medications are currently the best way to "manage" AD; however, there is no cure [5]. In this study, genetic and pharmacological tools are used to test the hypothesis that post-injury inhibition of reactive synaptogenesis will block the onset or reduce the severity of AD. After CNS injury, astroglia and macrophages secrete thrombospondins (TSP), a family of matricellular proteins that regulate cell-cell and cell-matrix interactions, most notably neurite growth and synaptogenesis [6, 7]. Eroglu et al. showed that astrocyte-derived TSPs cause synaptogenesis by binding to neuronal α2δ-1 receptors and that transgenic over expression of neuronal α2δ-1dramatically increases synaptogenesis [7]. TSP-4 is selectively increased in astrocytes surrounding injured spinal cord axons. Here with genetic tools, we predict that TSP-4 KO will block the onset or reduce the severity of AD, however the α2δ-1 over-expression will enhance the development of AD. Anti-epileptic/anti-neuropathic pain drugs gabapentin (Neurontin) and pregabalin (Lyrica) bind with α2δ-1 [8] thereby blocks TSP/α2δ-1 interactions as well as inhibits TSP-induced new synapse formation[7]. We hypothesize that inhibiting TSP binding to neuronal α2δ-1 with GBP will reduce the severity and frequency of AD by inhibiting maladaptive synaptogenesis after SCI. Our results indicated that mice carry an extra α2δ-1 gene developed more AD than WT littermates after T3 SCI. GBP s.c. injection inhibits AD development in WT BL6 mice. This study suggested that post-injury synapgenesis is an important mechanism underlining the development of AD post T3 SCI.

KYWORDS

Spinal cord injury (SCI), Spontaneous autonomic dysreflexia (sAD), induced autonomic dysreflexia (iAD), Thrombospondin (TSP), α2δ-1 receptor, Gabapentin (GBP), days post injury (DPI), common carotid artery (CCA), mean artery blood pressure (MABP), mean heart rate (MHR)

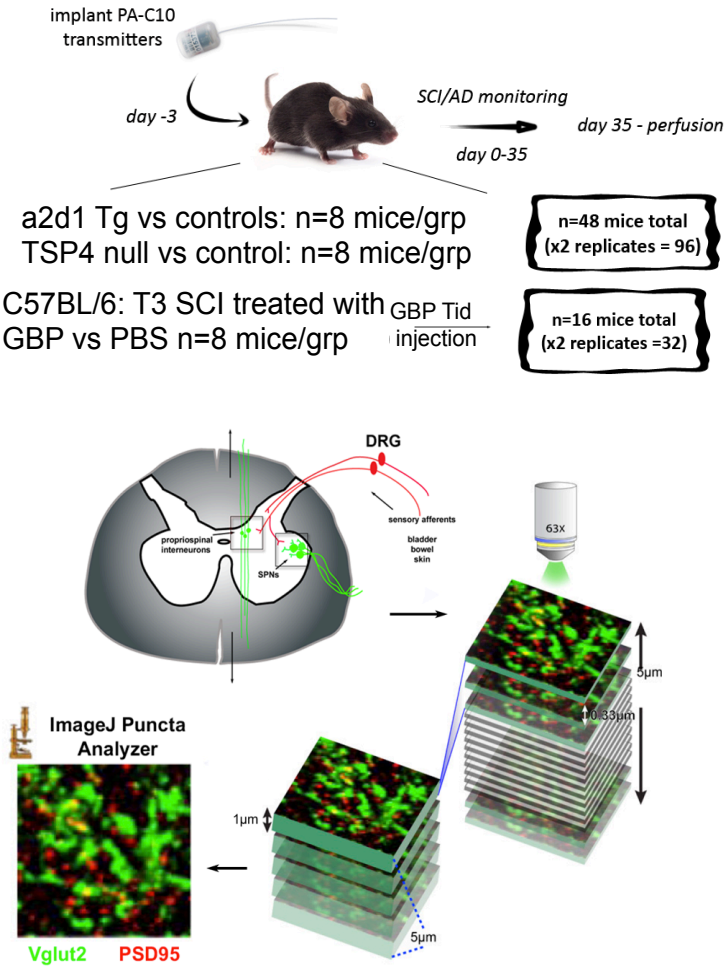
ACCOMPLISHMENTS

What were the major goals of the project?

Goal 1: Comparing SCI. AD events in the two mice strains (α2δ-1 TG; TSP null) vs their respective littermate after T3 (100% completion)

Goal 2: Comparing AD events in GBP vs control group after T3 SCI. (70% completion)

Goal 3: Complete immunohistochemistry analysis for synaptogenesis of all animals. (40% completion)



Experimental outline:
Goal 1: Comparing SCI. AD events in the two mice strains (α2δ-1 TG; TSP null) vs their respective littermate after T3 (100% completion)
Goal 2: Comparing AD events in GBP vs control group after T3 SCI. (70% completion)
Goal 3: Complete immunohistochemistry analysis for synaptogenesis of all animals. (40% completion)

What was accomplished under these goals?

Goal 1: Comparing AD events in two mice strains (α2δ-1 TG; TSP null) vs their respective littermates after T3 SCI. (100% completion)

1) Major activities and Specific objectives:

We obtained α2δ-1 TG (129SVE background) and TSP null mice (C57BL/6 background) lines from Dr Eroglu (Duke) on Feb 7, 2014. In the August 2014 (1st) and Oct 2015 (repeat), we bred enough α2δ-1 TG and WT littermates for first objective: comparing the development of AD (frequency and/or severity) in α2δ-1 TG vs WT littermates after T3 SCI. And in the July 2015 (1^{st}) and July 2016 (2^{nd} repeat), we bred enough TSP4 KO and WT littermates for the second objective: comparing the development of AD (frequency and/or severity) in TSP KO and WT littermates.

Surgery procedures: All animals were maintained in a pathogen-free environment before surgical implant of telemetry transmitters. Telemetry transmitters were implanted into each mouse as described previously [9]. Briefly, the PhysioTel telemetry system with PA-C10 telemetry transmitters (Data Sciences International) were implanted in anesthetized mice 3 days before SCI via a cannulation of the left common carotid artery (CCA) to monitor the blood pressure (BP) and heart rate (HR). The extra-vascular portion of the transmitter was placed into a subcutaneous pocket created on the lateral flank. The CCA was exposed through a midline incision on the neck, and the catheter of transmitter was introduced into the CCA through a small incision near the carotid bifurcation and advanced until the sensing region of the catheter was positioned in the aortic arch (8–9mmfrom the carotid bifurcation). Complete spinal cord transaction injuries were performed as described previously [10]. Briefly, using aseptic technique, a partial laminectomy was performed at vertebral level T3, after which the periosteum and dura mater were carefully opened. Using iridectomy scissors together with gentle aspiration, the spinal cord was cut, creating a clear separation between the rostral and caudal stumps of transected spinal cord. After injury, muscle and skin were sutured separately and then mice were injected with sterile saline (2 ml, s.c.) and placed individually into warmed HEPA filtered cages. Postoperative care included manual bladder expression 2/d and daily antibiotics for the first 7day post injury (dpi) (gentocin, 5mg/kg, s.c.). Dehydration was monitored daily and body weight and urinary pH was monitored weekly. All surgical procedures were approved by and performed in accordance with the Institutional Laboratory Animal Care and Use Committee at The Ohio State University.

Analysis of spontaneous AD: Dataquest data acquisition software (Data Sciences International) was used to acquire HR and BP data between 5 and 35 dpi at 5 s intervals with implanted telemetry transmitters. MATLAB software was used to create an algorithm that would detect episodes of spontaneous AD. The current automated program works similarly to the previous semi-automated method developed by Yi Zhang [9], but incorporates the manual validation steps into the computer algorithm to improve objectivity and efficiency as described in details in previous annual report.

Colorectal distension and pinch to intentionally elicit AD: Colorectal distension and cutaneous pinch was used to elicit spinal autonomic reflexes as described previously [9]. The tips of Hartman hemostats were shielded with polyethylene tubing and then used to pinch the flank below the level of SCI just rostral to the hip joint. To ensure consistent pinch intensity and duration, the hemostat was closed to the first click in every trial for 30 s. Colorectal distension was accomplished using a 4-French, 60 mm balloon-tipped catheter (Swan-Ganz monitoring catheter model 116F4; Edwards Life Sciences). The catheter was inserted into the anus, positioning the balloon ~1.5 cm from the anal opening and then securing the catheter to the tail with surgical tape. After securing the catheter, animals were left alone to acclimate for at least 20 min. To elicit AD, the balloon was inflated with 0.3 ml of air for 1 min. Distention was maintained for 1 min and repeat stimulation occurred after a 30 min rest. Peak changes in BP and corresponding HR were obtained and then compared with baseline values.

2) Significant results or key outcomes

Automated detection of AD events was established use a MATLAB program and validated by comparing with the semi-automated techniques (data shown in previous annual report). With this automated detection method we can easily analyze continuous cardiovascular data (e.g., MBP and MHR) over 35 days for 16 mice (limited by the number of transmitter receiver) within hours (as compared to weeks or months with previous method). Overall, the automated program works similarly to the previous semi-automated method, but incorporates the manual validation steps into the computer algorithm to improve objectivity and efficiency.

Both a2d1 TG and WT littermates, which are on a 129 genetic background, did not develop late

phase autonomic dysreflexia as described previously in WT C57BL/6J (B6) mice. Also, when compared with WT littermate control mice, the number of spontaneously occurring AD events increases significantly in a2d1 TG mice during the first week post-injury after T3 SCI (Figure 1). These data indicate genetically-encoded differences in mechanisms controlling autonomic dysfunction after SCI.

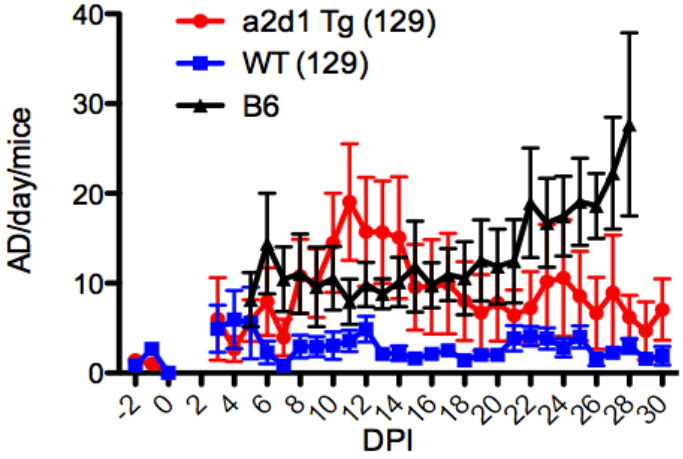


Figure 1. Spontaneous AD develops after SCI in a2d-1 transgenic (Tg) and WT littermate. AD is induced in a2d1Tg mice from 8-14DPI. Not like BL6 mice, late phase spontaneous AD does not develop in both a2d1 Tg and WT

To determine whether it is possible to intentionally elicit AD in 129 mice, skin pinch or colorectal distension were used to elicit somatic or visceral-sympathetic reflexes below the level of injury. Such stimuli are potent induces of autonomic dysreflexia after high-level SCI. Surprisingly, neither stimulus elicited AD in most (~80%) 129 mice (Figure 2).

Previous data from our lab indicate that high-level SCI (e.g., T3 injuries) causes immune suppression, an effect that is linked to the development of aberrant autonomic reflexes in the isolated spinal cord below the level of injury [9]. Although we were unable to elicit AD in 129 mice, we next determined whether immune suppression develops in these mice after T3 SCI. The spleens of both a2d1 TG, and WT mice were isolated at 38 days post-injury and spleen weights were normalized to body weight. The data are consistent with our previous observations in C57BL/6 SCI mice, i.e., there is significant splenic atrophy after T3 SCI in both a2d1 TG and WT 129 animals [9] (Figure 3). From these data we conclude that post-injury plasticity in the autonomic circuitry responsible for AD and immune suppression can be mutually exclusive.

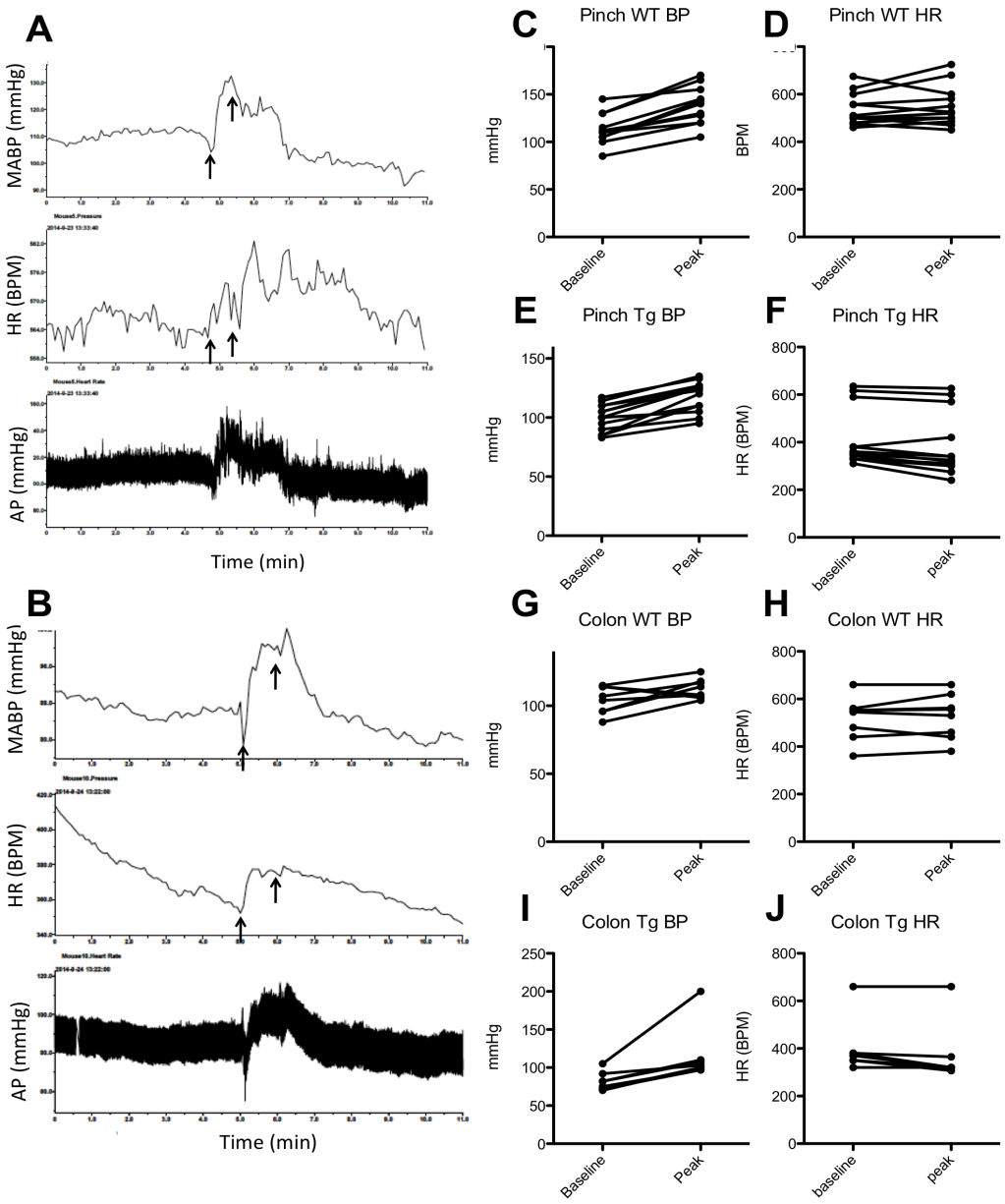


Figure 2. Cutaneous pinch and colorectal distension do not induce AD in the 129 mice. Representative pulsatile arterial pressure traces after applying colorectal distension (A) or cutaneous pinch (B) in mice at 35 DPI. Under each trace, MABP and mean HR changes are quantified showing before and after cutaneous pinch in WT (C, D) and a2d1 TG mice (E, F); or before and after colorectal distension in WT (G, H) and a2d1 TG mice (I, J). Arrows on each trace indicate the start and stop, respectively, for each stimulus. Quantification of MABP and mean HR changes in response to skin pinch (C-F) or colorectal distension (G-J). (Pinch: cutaneous pinch; Colon: colorectal distension)

To test the effect of TSP4 on post injury synaptogenesis and AD development, in July 2016, we repeated a previous experiment in which we analyzed AD development after T3 SCI in TSP4 KO and WT littermate control mice. The data indicate that there are no significant differences in AD development between TSP4KO and WT littermate control mice. Below, we report data from the first experiment (Figure 4A) and the repeat experiment (Figure 4B). Figure 4C combines the data from both independent studies.

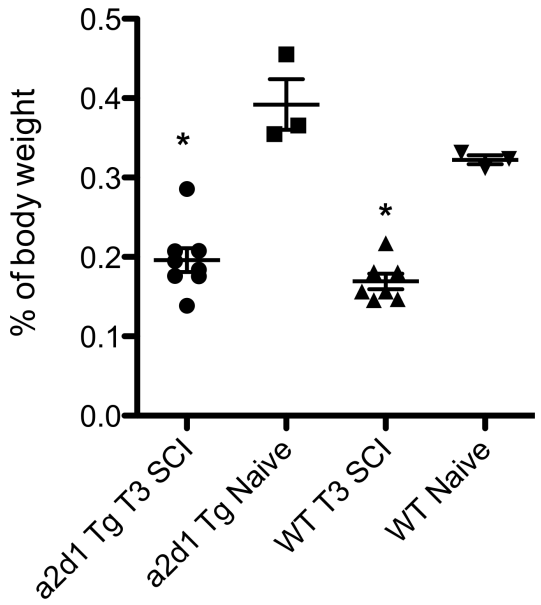


Figure 3. Spleen weight is significantly reduced at 38 dpi in both WT and a2d1 Tg mice n=8-3 for a2d1 Tg mice and n=7-3 for WT; *p<0.01 comparing with Naïve mice.

Note, that in both studies, WT mice showed an early peak of AD (second week post-injury); however, there were no significant differences between groups over time. Also, we noted inter-experimental differences in the overall magnitude of AD events. Such differences are difficult to explain but we did note that there were age and sex differences between mice used in Exp 1 and Exp. 2 (Figure 4D). Such differences were unavoidable as mice were used as they became available from breeding pairs. Still, the average age of mice used in the first experiment was ~6 months while those in Exp. 2 were ~3 months. Also, 56% of mice in Exp. 1 were male while 50% of mice were male in Exp 2. Whether age and sex differences contribute to differences in the magnitude of early AD events is not known and is outside the scope of the funded studies.

To determine if TSP KO affects intentionally induced AD (iAD), a skin pinch or colorectal distension (CRD) stimulus was used to elicit a somatic or visceral-sympathetic reflex below the level of injury in TSP KO and WT littermates at 30dpi. These stimuli potently induce AD after high-level (T3) SCI. Data indicates that both stimuli elicit AD in both groups with similar level increase in BP with a corresponding decrease in HR (Figure 5).

Previous data from our lab indicate that high-level (T3) SCI causes splenic atrophy and immune suppression, an effect that is linked to the development of aberrant autonomic reflexes in the isolated spinal cord below the level of injury. Accordingly, we measured spleen weight in TSP4 KO and WT mice isolated at 30 dpi. Data show that there is no significant difference in spleen weights (Figure 6).

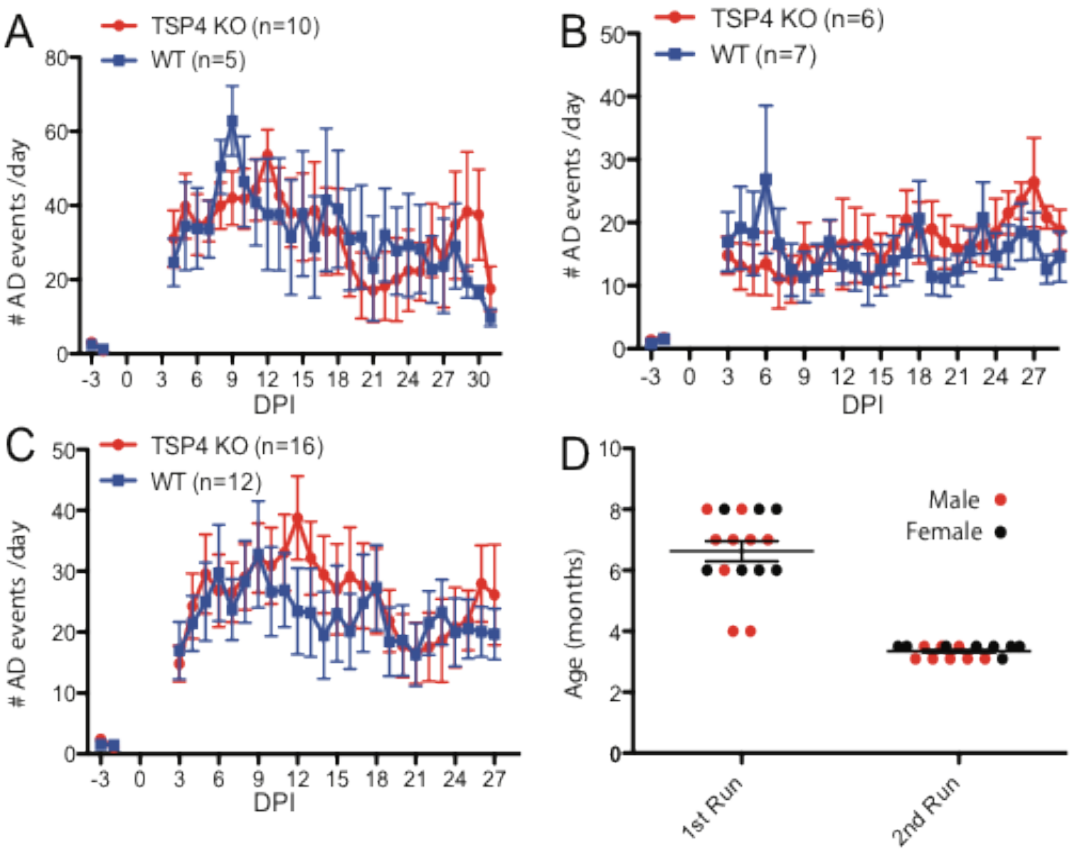


Figure 4. Spontaneous AD (sAD) develops after SCI in TSP4 KO and WT littermate at similar level in two independent experiments. sAD develops in both TSP4KO and WT littermate control mice. Independent replicate experiments (first run - A; second run - B) and combined data (C) are shown. There were differences in sex and age between the replicate experiments (D) which could explain differences in the frequency of AD events in the acute post-injury phase (compare #AD events in A and B).

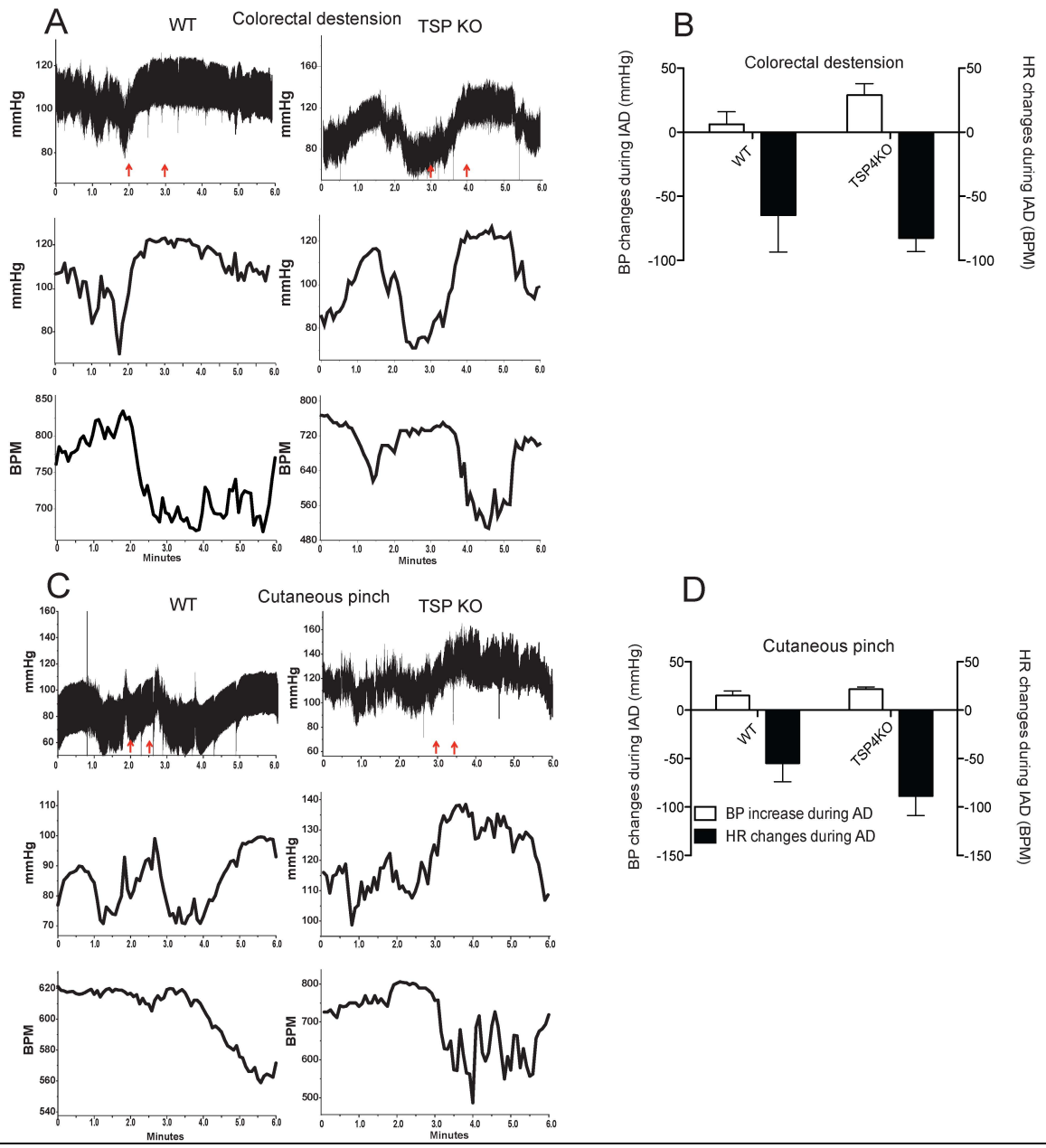


Figure 5. Colorectal distension (A, B) and cutaneous pinch (C, D) increase blood pressure (BP) and decrease heart rate (i.e., elicit AD) in both TSP4 KO and WT littermates. Representative raw BP tracing, Mean artery blood pressure (MABP) and mean heart rate (MHR) changes are showed after 1min colon extension (A) and 30 seconds subcutaneous pinch (C) (red arrows indicates the beginning and ending of the stimulation). The accumulative data for MBP increase and MHR decrease after colorectal distension and cutaneoue pinch in WT and TSP KO mice are showed in B and D.

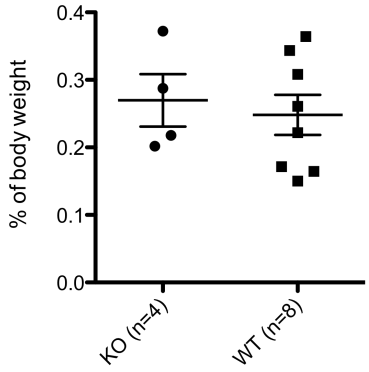


Figure 6. There is no significant difference in spleen size between TSP4 KO and WT littermates at 30 DPI. n=4-8

3) Other achievements.

Nothing to report.

Goal 2: Comparing AD events in GBP vs control group after T3 SCI. (70% completion)

1) Major activities and Specific objectives:

We tested whether high dose gabapentin (GBP) (200mg/kg) TID can reduce post-injury synaptogenesis and the frequency and/or severity of AD in Feb 2016.

2) Significant results or key outcomes

To test if high dose of gabapentin (GBP) inhibits the post injury synaptogenesis and the development of AD after T3 SCI, GBP (200mg/kg) or 100ul PBS was injected s.c. 3 times / day (TID) after T3 SCI for 4 weeks. Data in Figure 7 shows that GBP reduces the frequency of AD.

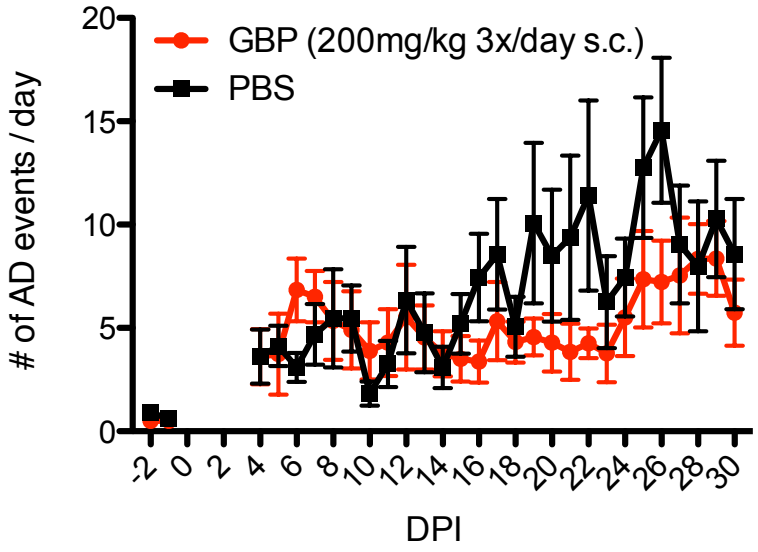


Figure 7. High dose GBP (200mg/kg, tid) reduces the frequency of spontaneous AD after T3 SCI. N=8/group

To determine if GBP affect intentionally-induced AD (iAD), a skin pinch or colorectal distension (CRD) stimulus was used to elicit a somatic or visceral-sympathetic reflex, respectively, below the level of injury in GBP and PBS treated mice at 30 DPI. These stimuli potently induce AD after high-level (T3) SCI. Data indicate that both stimuli elicit AD in PBS-treated mice, as defined by an increase in systemic BP with a corresponding decrease in HR. However iAD could not be elicited in GBP-treated mice (Figure 8).

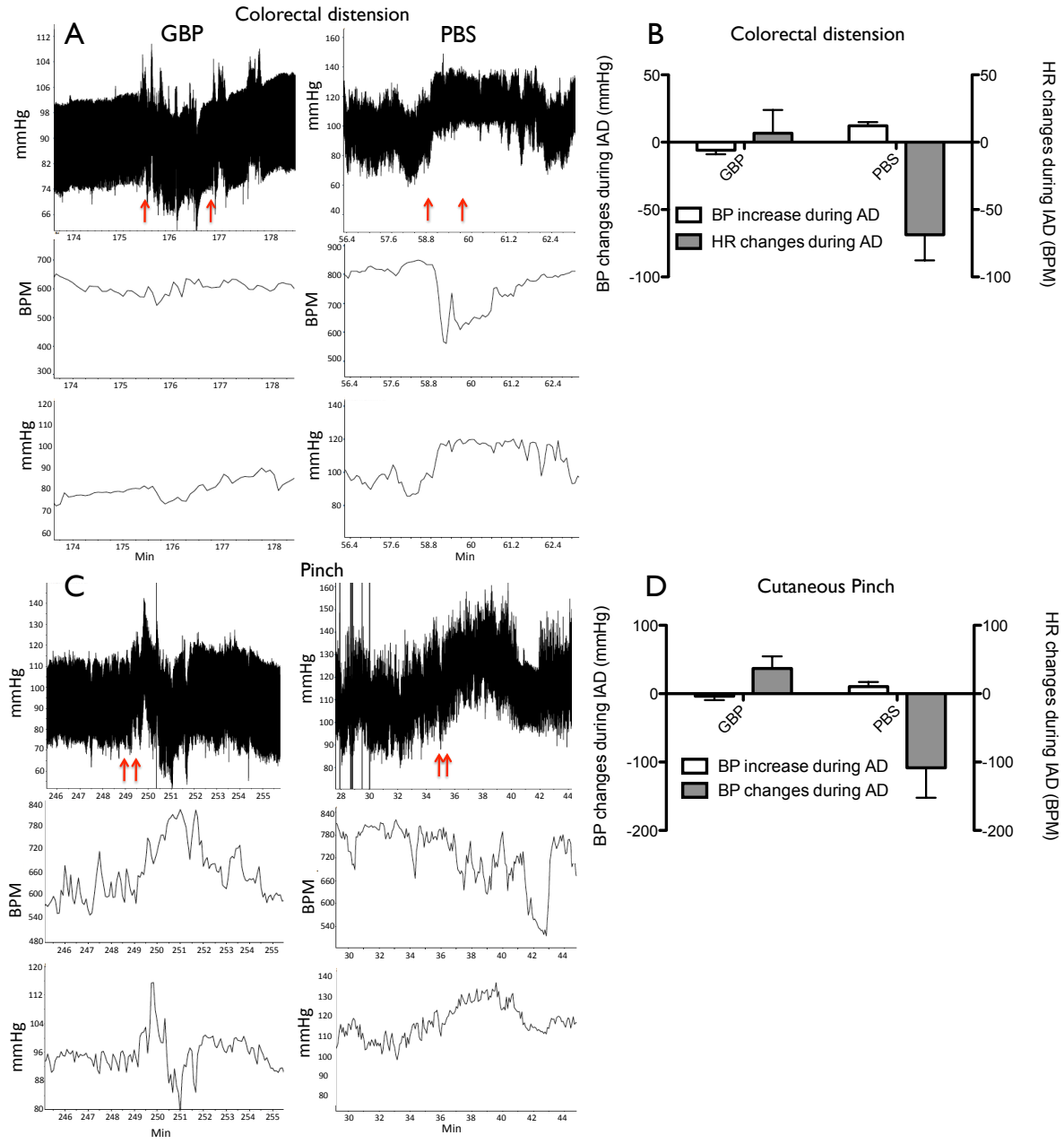


Figure 8. Cutaneous pinch and colorectal distension do not induce AD in the GBP treated mice. Representative pulsatile arterial pressure traces after applying colorectal distension (A) or cutaneous pinch (C) in mice at 30 DPI. Mean artery blood pressure (MABP) and mean heart rate (MHR) changes are showed after 1min colon extension (A) and 30 seconds subcutaneous pinch (C) (red arrows indicates the beginning and ending of the stimulation). The accumulative data for MBP increase and MHR decrease after colorectal distension and cutaneous pinch in PBS and GBP treated mice are showed in B and D.

To further test if GBP treatment has causes effect on T3 SCI-induced splenic atrophy and immune suppression, we measured spleen weight from GBP and PBS treated mice isolated at 30

dpi. Data show that high dose chronic GBP treatment significantly reduced spleen atrophy caused by T3 SCI (Figure 9).

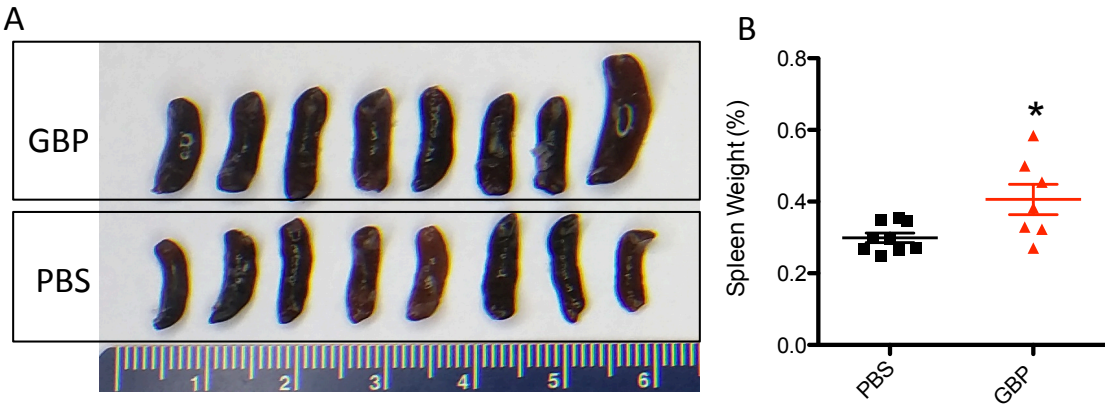


Figure 9. High dose GBP (200mg/kg s.c. TID) significantly reduced splenic atrophy measured at 30 days after SCI. Individual spleens from GBP or PBS-treated SCI mice (A). Spleen weight show as % of total body weight from PBS and GBP-treated SCI mice. Student's T test $p<0.05$, n=7 (GBP) n=9 (PBS).

3) Other achievements.

Nothing to report.

Goal 3: Complete immunohistochemistry analysis for synaptogenesis of all animals. (30% completion)

1) Major activities and Specific objectives:

All mice (a2d1 TG vs WT; TSP4 KO vs WT, GBP 200mg/kg s.c. tid vs PBS), after recording ~30 days of BP and HR, were perfused as described previously[14]. In detail, mice were anesthetized intracardially perfused with TBS (25mM Tris-base, 135 mM NaCl, 3mM KCl, pH7.6) supplemented with 7.5 uM heparin followed with 4% PFA in TBS. The brains and spinal cord were removed and fixed with 4% PFA in TBS at 4C overnight. The next day, Tissue were cryoprotected with 30% sucrose in TBS until tissue sink. All samples were blinded by genotype or treatment. Total of 63 spinal cords and 63 brains (16 samples from GBP vs PBS; 14 samples from TSP4KO vs WT; 33 samples from a2d1 TG vs WT) were shipped to Dr Eroglu (Duke University) for analysis for the synapse genesis. The samples were given number and letter identifiers by the Popovich lab and Eroglu Lab members remained blinded to genotype and/or manipulation until after analyses.

For synaptic puncta analysis, PFA-fixed brains or spinal cord were cryoprotected with 30% sucrose in TBS overnight and were then embedded in a 2:1 mixture of 30% sucrose in TBS:OCT (Tissue-Tek, Sakura, Japan). Brain and spinal cord were cryosectioned (coronal) at 20 µm using Leica CM3050S (Leica, Germany). Sections were washed and permeabilized in TBS with 0.2% Triton-X 100 (TBST; Roche, Switzerland) 3 times 10min at room temperature. Sections were blocked in 5% Normal Goat Serum (NGS) in TBST for 1 hour at room temperature. Primary antibodies (guinea pig anti-VGlut1 1:3500 (AB5905, Millipore, MA), rabbit anti-PSD95 1:300 (51-6900, Invitrogen, CA)) were diluted in 5% NGS containing TBST. Sections were incubated overnight at 4°C with primary antibodies. Secondary Alexa-fluorophore conjugated antibodies (Invitrogen) were added (1:200 in TBST with 5% NGS) for 2 hours at room temperature. Slides were mounted in Vectashield with DAPI (Vector Laboratories, CA) and images were acquired on a Leica SP5 confocal laser-scanning microscope. Three animals per group were analyzed, with three independent cross sections per each mouse. Five µm thick confocal z-stacks (optical section depth 0.33µm, 15 sections/z-stack, imaged area/scan=20945 μm^2) were imaged at 63x magnification on a Leica SP5 confocal laser-scanning microscope. Maximum projections of 3 consecutive optical sections (corresponding to 1 µm total depth) were generated from the original z-stack. Analyses were performed blind as to genotype. The Puncta Analyzer plugin that was developed by Barry Wark (available upon request) for ImageJ 1.29 (NIH; http://imagej.nih.gov/ij/, version ImageJ 1.29 is available at http://labs.cellbio.duke.edu/Eroglu/Eroglu_Lab/Publications.html) was used to count the number of co-localized, pre-, and post-synaptic puncta.

2) Significant results or key outcomes:

The preliminary results from 3 pairs of the SCI mice with GBP (200mg/kg s.c. tid) treatment vs PBS injection suggested GBP treatment did not reduce the excitatory synapse (Figure 10) as we expected but increase the amount of inhibitory synapse (Figure 11). Excitatory (VGlut2/Homer) and inhibitory (VGAT/Gephyrin) synaptic staining in two regions: T1-T3 (site above injury) and lower down at T4-T9 (sit below the injury). There is not any change in the excitatory synapses (although Homer itself was increased with GBP in both regions) (Figure 10). However, there was a dramatic increase in inhibitory synapses in both regions with GBP (Figure

11). This was driven by up-regulation of both VGAT and gephyrin. GBP may improve sprouting of inhibitory neuron axons and that results in rewiring of inhibitory circuits.

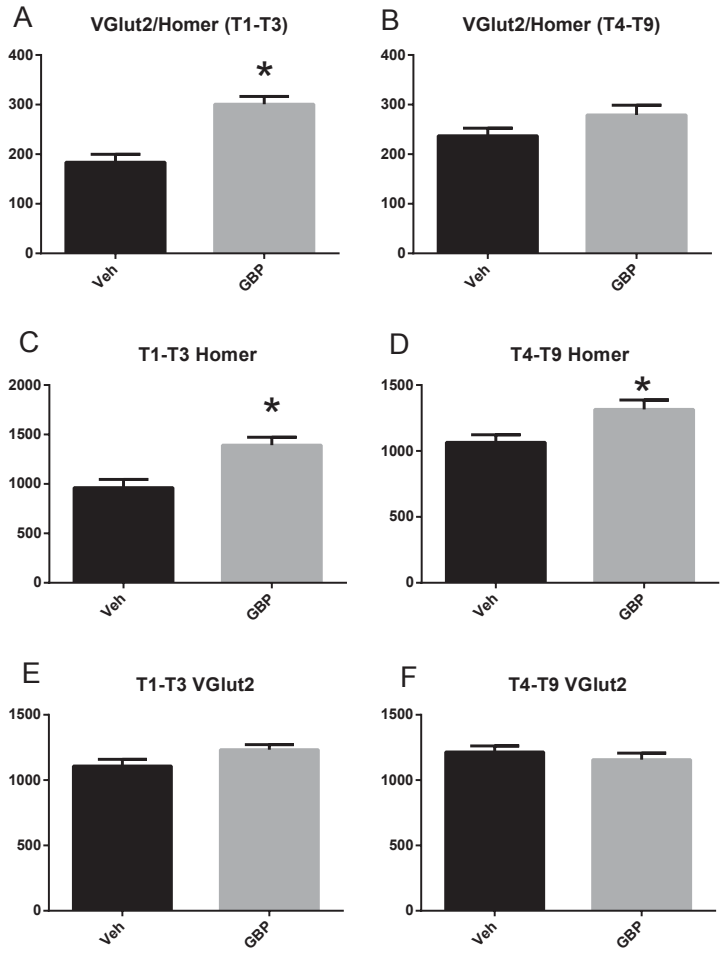


Figure 10. GBP treatment increase excitatory synapse (Vglut2/Homer) rostral (A) but not caudal (B) side of the lesion after 30 days post T3 SCI. Cross sections of spinal cord at level of T1-T3 and T4-T9 were stained with Homer (an excitatory postsynaptic marker) and VGlut2 (an excitatory presynaptic marker). The excitatory synpatic puncta co-labeled with both VGlut2 and Homer were quantified with Image J (A, B). The excitatory postsynapses were count with Image J (C, D). The excitatory presynapses were quantified also (E, F). n=3 *$p<0.05$ with Student's T-test.

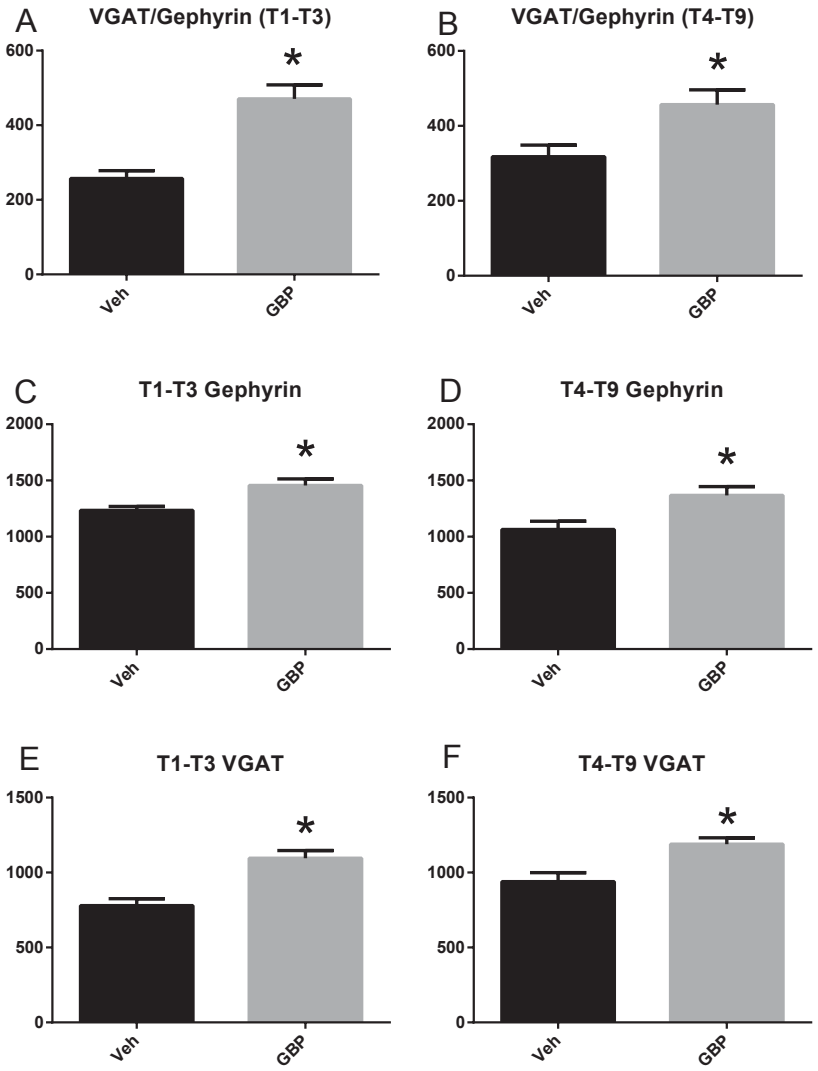


Figure 11. GBP treatment increase inhibitory synapse (VGAT/Gephyrin) both rostral (A) and caudal (B) sides of the lesion after 30 days post T3 SCI. Cross sections of spinal cord at level of T1-T3 and T4-T9 were stained with Gephyrin (an inhibitory postsynaptic marker and) and VGAT (an inhibitory presynaptic marker). The excitatory synpatic puncta co-labeled with Gephyrin and VGAT were quantified with Image J (A, B). The inhibitory postsynapses were labeled with Gephyrin and quantified (C, D). The inhibitory presynapses were labeled with VGAT and quantified (E, F). n=3 *p<0.05 with Student's T-test.

3) Other achievements.

In April 2016, Dr Eroglu group tested the impact of spinal cord injury on synaptic density in the brain, Pre- and post-synaptic puncta in sagittal sections from Black6 injured mice and sham controls were measured with IHC. Primary motor cortex (M1) and various nuclei in the brainstem that send direct projections to the spinal cord (locus coeruleus [LC], subcoeruleus [SC], and group A5) and one that does not project to spinal cord (nucleus of the solitary tract [NTS]) were analyzed (Fig. 12A). In the motor cortex, the predominant excitatory inputs can be labeled with vesicular glutamate transporter 1 (VGlut1). Co-localization of VGlut1 with postsynaptic homer was not significantly affected in injured mice compared to their control counterparts (Fig. 12B). VGlut1 is not as highly expressed in the brainstem as in the cortex; however, analysis of the brainstem revealed a significant decrease in VGlut1/homer puncta in

injured mice (Fig. 12C). Like VGlut1, a second excitatory synapse marker, VGlut2, failed to show any differences in injured M1 cortex (Fig. 12D). Instead, VGlut2 showed significantly reduced co-localization with homer throughout the brainstem (Fig. 12E), where it is normally very highly expressed. These results confirm that a significant synaptic re-wiring of the brainstem occurs following spinal cord injury.

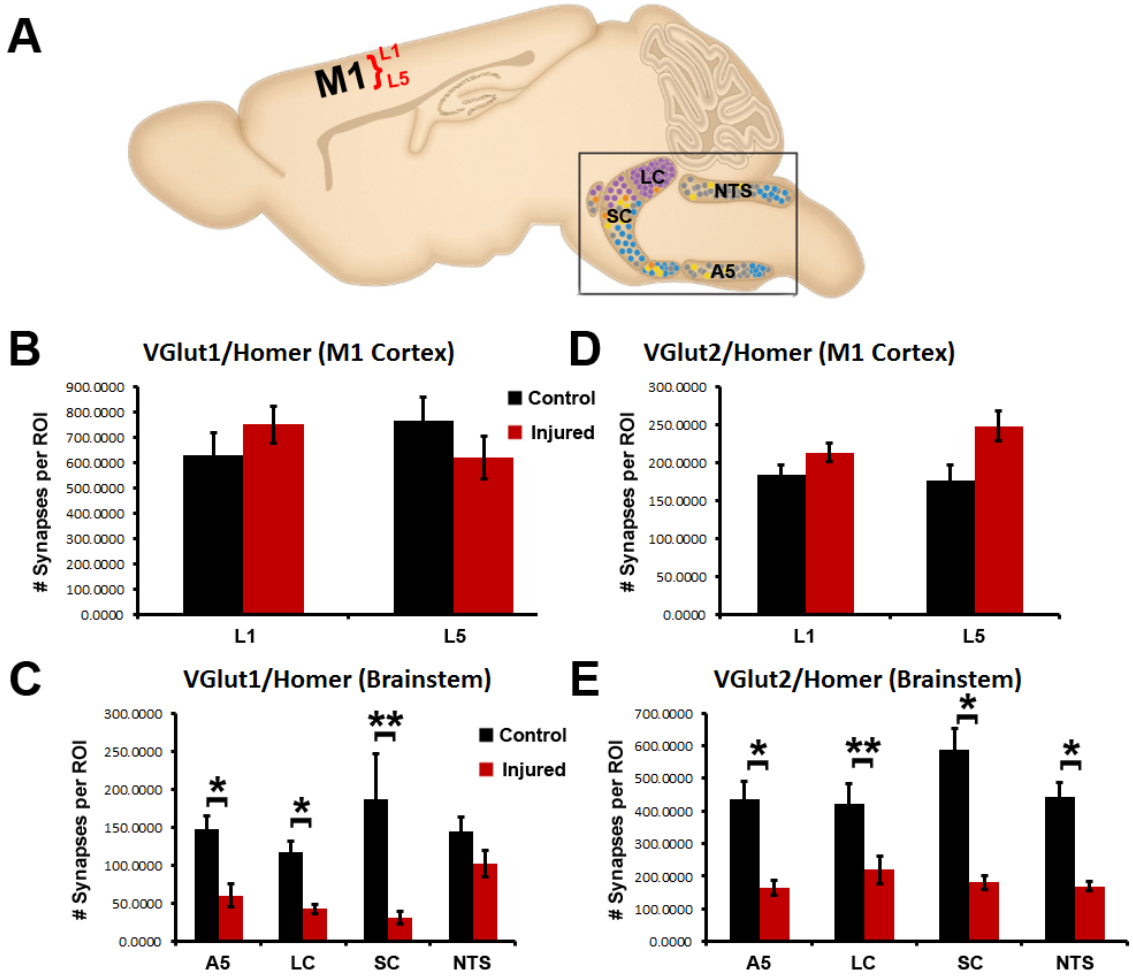


Figure 12 Spinal cord injury impacted synaptic density in the brain. (A) Schematic representation of a sagittal section from mouse brain. Regions used for analysis include primary motor cortex (M1; layers 1 and 5 indicated in red) and the brainstem (locus coeruleus [LC]; subcoeruleus [SC]; group A5; nucleus of the solitary tract [NTS]). Adapted from Robertson et al., Nat Neurosci 2013. (B) Co-localized synaptic puncta (presynaptic VGlut1/postsynaptic Homer) are not significantly different between control and injured mice in M1 (for all analyses in brain: n=3 mice per condition; analyzed 3 sections per region from each mouse). (C) VGlut1/Homer synaptic puncta are significantly decreased in injured mice compared to uninjured mice for all brainstem regions analyzed except for NTS (*p<0.01; *p<0.05; student's T-test). (D) VGlut2/Homer synapses in M1 are unaffected in injured mice. (E) Injured mouse brainstem shows a significant reduction in VGlut2/Homer synapses across all regions analyzed (*p<0.01; *p<0.05; student's T-test).

What opportunities for training and professional development has the project provided?
Nothing to Report
How were the results disseminated to communities of interest?
Nothing to Report
What do you plan to do during the next reporting period to accomplish the goals?
For the next reporting period, we plan to repeat whether prophylactic gabapentin (GBP) can reduce post-injury synaptogenesis and the frequency of AD.

We will continue the Spinal cord synapse analysis for all experiments (a2d1 vs WT; TSP4 vs WT, GBP vs PBS treatment).

IMPACT

What was the impact on the development of the principal disciplines of the project?

Our preliminary study suggested high dose of GBP treatment reduce the frequency of AD after T3 SCI may caused by the increase of inhibitory synapse formation instead of reduce the excitatory synapse which is a novel finding and should be further tested.

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

CHANGES/PROBLEMS

Changes in approach and reasons for change

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

Changes that has a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals biohazards, and/or select agents

Nothing to report

Significant changes in use or care of human subjects

NA

Significant changes in use or care of vertebrate animals

NA

Significant changes in use of biohazards and/ or select agents.

NA

PRODUCTS

Publications, conference papers, and presentations

Nothing to report

Website or other Internet site

Nothing to report

Technologies or techniques

An autonomic AD detection algorithm was developed in this study.

Invention, patent applications, and/or licenses

Nothing to report

Other products

Nothing to report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Yan Wang
Project Role:	Postdoc Researcher
Researcher Identifier	200143183
Nearest person month worked	12
Contribution to project:	Performing experiment, data analysis
Funding support:	
Name:	Zhen Guan
Project Role:	Senior Research Associate
Researcher Identifier	
Nearest person month worked	12
Contribution to project:	Performing experiment
Funding support:	
Name:	J. Hayes Davis
Project Role:	Undergraduate Researcher
Researcher Identifier	2003187666
Nearest person month worked	10
Contribution to project:	data analysis
Funding support:	
Name:	Phillip Popovich
Project Role:	Professor
Researcher Identifier	
Nearest person month worked	12
Contribution to project:	Design the experiment, instruction.
Funding support:	

Has there been a change in the active other support of the PI or key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

Organization Name: Duke University Medical Center Department of Cell Biology
Location: 334 Nanaline Duke Building, Durham, NC 27710
Collaboration: Dr. Cagla Eroglu, Ph.D. collaborate with us on the project

SPECIAL REPORTING REQUIREMENTS

Collaborative awards:

For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to https://ers.amedd.army.mil for each unique award

QUAD CHARTS:

If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

Attached in the end.

APPENDICES

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc

The AD detecting algorithm is attached in the end.

1. **Weaver, LC, Fleming, JC, Mathias, CJ, and Krassioukov, AV. Disordered cardiovascular control after spinal cord injury. *Handb Clin Neurol* 109: 213-233.**
2. **Krassioukov, A, and Claydon, VE (2006). The clinical problems in cardiovascular control following spinal cord injury: an overview. *Prog Brain Res* 152: 223-229.**
3. **Rabchevsky, AG (2006). Segmental organization of spinal reflexes mediating autonomic dysreflexia after spinal cord injury. *Prog Brain Res* 152: 265-274.**
4. **Hou, S, Duale, H, Cameron, AA, Abshire, SM, Lyttle, TS, and Rabchevsky, AG (2008). Plasticity of lumbosacral propriospinal neurons is associated with the development of autonomic dysreflexia after thoracic spinal cord transection. *J Comp Neurol* 509: 382-399.**
5. **Rabchevsky, AG, and Kitzman, PH. Latest approaches for the treatment of spasticity and autonomic dysreflexia in chronic spinal cord injury. *Neurotherapeutics* 8: 274-282.**
6. **Christopherson, KS*, et al.* (2005). Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. *Cell* 120: 421-433.**
7. **Eroglu, C*, et al.* (2009). Gabapentin receptor alpha2delta-1 is a neuronal thrombospondin receptor responsible for excitatory CNS synaptogenesis. *Cell* 139: 380-392.**
8. **Gee, NS, Brown, JP, Dissanayake, VU, Offord, J, Thurlow, R, and Woodruff, GN (1996). The novel anticonvulsant drug, gabapentin (Neurontin), binds to the alpha2delta subunit of a calcium channel. *J Biol Chem* 271: 5768-5776.**
9. **Zhang, Y*, et al.* Autonomic Dysreflexia Causes Chronic Immune Suppression after Spinal Cord Injury. *J Neurosci* 33: 12970-12981.**
10. **Lucin, KM, Sanders, VM, Jones, TB, Malarkey, WB, and Popovich, PG (2007). Impaired antibody synthesis after spinal cord injury is level dependent and is due to sympathetic nervous system dysregulation. *Exp Neurol* 207: 75-84.**
11. **Constant, I, Laude, D, Elghozi, JL, and Murat, I (2000). Assessment of autonomic cardiovascular changes associated with recovery from anaesthesia in children: a study using spectral analysis of blood pressure and heart rate variability. *Paediatr Anaesth* 10: 653-660.**
12. **Inoue, K*, et al.* (1995). Assessment of autonomic function in traumatic quadriplegic and paraplegic patients by spectral analysis of heart rate variability. *J Auton Nerv Syst* 54: 225-234.**
13. **Goa, KL, and Sorkin, EM (1993). Gabapentin. A review of its pharmacological properties and clinical potential in epilepsy. *Drugs* 46: 409-427.**
14. **McKinstry, SU*, et al.* Huntingtin is required for normal excitatory synapse development in cortical and striatal circuits. *J Neurosci* 34: 9455-9472.**

15. van Bogaert, MJ, Groenink, L, Oosting, RS, Westphal, KG, van der Gugten, J, and Olivier, B (2006). Mouse strain differences in autonomic responses to stress. *Genes Brain Behav* 5: 139-149.

16. Campen, MJ, Tagaito, Y, Jenkins, TP, Balbir, A, and O'Donnell, CP (2005). Heart rate variability responses to hypoxic and hypercapnic exposures in different mouse strains. *J Appl Physiol (1985)* 99: 807-813.